PRIORITY
04-20-92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
WALLACH et al)
Serial No.: 07/625,668)
Filed: December 13, 1990)
For: EXPRESSION OF THE RECOMBINANT TUMOR NECROSIS FACTOR BINDING...)

Atty's Docket: WALLACH=4
Art Unit: 184
Examiner: Bugaisky, G.

Washington, D.C.

April 6, 1992

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REQUEST FOR PRIORITY

Honorable Commissioner of Patents and Trademarks
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Sir:

In accordance with the provisions of 37 CFR 1.55 and the requirements of 35 USC 119, there is filed herewith a certified copy of:

	Israel	Appln. No.:	095064	Filed:	December 7, 1990
and	Israel	Appln. No.:	092697	Filed:	December 13, 1989

It is respectfully requested that applicant be granted the benefit of the priority date of the foreign application.

Respectfully submitted,

BROWDY AND NEIMARK
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Enclosures
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#10

STATE OF ISRAEL

This is to certify that annexed hereto is a true copy of the documents as originally deposited with the patent application particulars of which are specified on the first page of the annex.

זאת לתעודה כי רצופים בזה העתקים נכונים של המסמכים שהופקדו לכתחילה עם הבקשה לפטנט לפי הפרטים הרשומים בעמוד הראשון של הנספח.

This 18 NOV 1990 היום

רשם הפטנטים
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מספר: Number	092697
תאריך: Date	13-12-1989
הוקדם/נדחה Ante/Post-dated	

חוק הפטנטים, תשכ"ז—1967
PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
I (Name and address of applicant, and in case of body corporate-place of incorporation)

Yeda Research and Development Co. Ltd.
A company registered under the laws of Israel

P.O. Box 95, Rehovot, Israel

ידע חברה למחקר ופיתוח בע"מ
חברה רשומה בישראל
ת.ד. 95
רחובות

בעל אמצאה מכח העברה מהממציאים ששמה הוא
Owner, by virtue of Assignment by the inventors of an invention the title of which is

(בעברית) (Hebrew) שיבוט מולקולרי של חלבון הקושר TNF

(באנגלית) (English) Molecular cloning of TNF Binding Protein

מבקש בזאת כי ינתן לי עליה פטנט
hereby apply for a patent to be granted to me in respect thereof.

• בקשת חלוקה — Application of Division	• בקשת פטנט מוסף — Application for Patent Addition	• דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	• לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
No. .... מס'	No. .... מס'			
dated .... מיום	dated .... מיום			

• יפוי כח: כללי / מיוחד — רצוף בזה / עוד יוגש
P.O.A.: general/individual-attached/to be filed later-
filed in case .... הוגש בענין

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היום 11 בחודש דצמבר שנת 1989
This 11 of December of the year 1989

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שיבוט מולקולרי של חלבון הקושר TNF

Molecular cloning of TNF Binding Protein

Yeda Research and
Development Co. Ltd.
T/811

The invention relates to Tumor Necrosis Factor (TNF) Binding Protein I and more particularly, to the cloning of gene coding for said protein.

Patent Application No. 83878 of the same applicant discloses a new protein found in urine and capable of inhibiting the binding of TNF to its receptors and the cytotoxic effect of TNF. This protein is now referred to hereinafter as TNF Binding Protein I or TBP-I.

The process for the extraction and purification of TBP-I in the above mentioned patent application comprises the following steps:

(a) recovering the crude protein fraction from a dyalized concentrate of human urine;

(b) subjecting said crude protein fraction of step (a) to ion exchange chromatography to obtain partially purified active fractions of the TNF Binding Protein defined by its ability to inhibit both the binding of TNF to its receptors and the cytotoxic effect of TNF;

(c) applying said partially purified active fractions of the TNF Binding Protein from step (b) to reversed phase high pressure liquid chromatography (HPLC) to obtain substantially purified active fractions of the TNF Binding Protein defined by its ability to inhibit both the binding of TNF to its receptors and the cytotoxic effect of TNF; and

(d) recovering the substantially purified protein of step (c), said protein having a molecular weight of about 26-28 Kda on SDS PAGE under reducing conditions, moving as a single peak on reversed phase HPLC and having the ability to inhibit both the binding of TNF to its receptors and the cytotoxic effect of TNF.

The purified TBP-I was sequenced and shown to contain at the N-terminus the following amino acid sequence:

1 5 10 15

Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-Asn-Asn-Ser

The invention relates to oligonucleotide probes to the cDNA coding for a protein comprising the amino acid sequence of TBP-1. The probes were synthesized by known methods on the basis of the above amino acid sequence of the N-terminus of TBP-I.

The invention also relates to a DNA molecule comprising a recombinant DNA molecule or a cDNA molecule coding for a protein comprising the amino acid sequence of TBP-I or a protein substantially homologous therewith. Within the scope of the invention are DNA molecules encoding said homologous proteins having the same biological activities of TBP-I.

In a preferred embodiment, the DNA molecule is a cDNA molecule picked up from a human cDNA library, in particular a colon cDNA library. Illustrated in Figure 1 is a partial restriction map of an insert of about 1.0 Kb of such a cDNA molecule obtained in agarose gel and herein designated C2. Figure 2 illustrates a partial nucleotide sequence of said C2 insert and also a partial translated amino acid sequence comprising the NH_2-terminal amino acid sequence of TBP-I encoded thereby. Figure 3 illustrates another partial nucleotide sequence of the C2 insert starting from nucleotide 342. Figure 4 shows a possible nucleotide sequence of the whole insert, that seems to have 965 nucleotides.

The invention further comprises cloning of said cDNA molecule

into a replicable plasmid vector and transformation of a bacterium, e.g., competent E.coli TG1 therewith.

In another aspect, the invention comprises the isolation of mRNA coding for a protein comprising the amino acid sequence of TBP-I by extraction from cells and its detection by hybridization with the cDNA of the invention.

Once the mRNA is obtained in a purified form, the cDNA coding for a protein comprising the amino acid sequence of TBP-I can be obtained by contacting the mRNA with reverse transcriptase for a time and under conditions sufficient to form said cDNA. This cDNA may be converted to double stranded cDNA by known techniques.

Probes may be prepared from the cDNA sequences of the invention and used for isolation of the genomic DNA coding for a protein comprising the amino acid sequence of TBP-I by known methods, e.g. by colony hybridization techniques under stringent conditions.

The DNA of positive clones are then inserted into appropriately constructed expression vectors by techniques well known in the art. Double-stranded cDNA is linked to plasmid vectors by homopolymeric tailing or by restriction linking involving the use of synthetic DNA linkers or blunt-ended ligation techniques. DNA ligases are used to ligate the DNA molecules and undesirable joining is avoided by treatment with alkaline phosphatase.

In order to be capable of expressing a desired protein, an expression vector should comprise also specific nucleotide sequences containing transcriptional and translational regulatory information linked to the DNA coding for the desired protein in such a way as to permit gene expression and production of the protein. The gene must be preceded by a promoter in order to be transcribed. There are a

variety of such promoters in use, which work with different efficiencies (strong and weak promoters).

The DNA molecule comprising the nucleotide sequence coding for a protein comprising the amino acid sequence of TBP-I preceded by a nucleotide sequence of a signal peptide and the operably linked transcriptional and translational regulatory signals is inserted into a vector which is capable of integrating the desired gene sequences into the host cell chromosome. The cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector.

In a preferred embodiment, the introduced DNA molecule will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Factors of importance in selecting a particular plasmid or viral vector include the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Once the vector or DNA sequence containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means: transformation, transfection, conjugation, protoplast fusion, electroporation, calcium phosphate precipitation, direct microinjection, etc.

Host cells to be used in this invention may be either prokaryotic or eukaryotic. Preferred prokaryotic hosts include bacteria, such as E.coli. Under such conditions, the protein will

not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

Preferred eukaryotic hosts are mammalian cells, e.g., human, monkey, mouse and chinese hamster ovary (CHO) cells, because they provide post-translational modifications to protein molecules including correct folding or glycosylation at correct sites. Also yeast and insect cells can carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene products and secretes peptides bearing leader sequences (i.e. pre-peptides).

After the introduction of the vector, the host cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of the desired protein or a fragment thereof. The expressed protein is then isolated and purified by any conventional procedure involving extraction, precipitation, chromatography, electrophoresis, or the like.

The invention will be illustrated by the following examples:

Example 1

Preparation of oligonucleotide probes

Oligonucleotide probes to the cDNA of TBP-I were designed on the basis of the NH_2-terminal amino acid sequence of the protein. Three mixtures of the synthetic oligonucleotides shown below and designated 1008, 1009, 1010 were used.

Probe 1008 is a mixture of 64 different 26-mers, in which deoxyinosine was introduced wherever the number of possible alternative codons for the amino acid exceeded 3. The two other mixtures are 17-mers; the first, probe 1009, is a mixture of 128 different oligonucleotides and the second, probe 1010, of 196 different oligonucleotides. Each of these two latter mixtures corresponds to part of the amino acid sequence coded-for by 1008. The nucleotide sequence of these two mixtures overlap each other.

```
1008      GGI GTC CCI TTC ATA TAA GTA GGI GT
                T       T   G   G   G
                                    T

1009      GGA GTC CCA TTC ATA TA
            C   T   C   T   G
            T       G
            G       T

1010                  TTC ATA TAA GTA GGA GT
                        T   G   G   G   C
                                T       G
                                        T
```

Example 2

Isolation of cDNA clones

cDNA clones comprising a nucleotide sequence coding for a protein comprising the amino acid sequence of TBP-I were isolated from a human cDNA colon library with the aid of the oligonucleotide probes of Example I as follows:

Four cDNA libraries constructed in lambda gt11 (Clontech Laboratories, Inc., U.S.A.) derived from the mRNA of human liver, human placenta, human colon and of HeLa cells were screened with the aid of the 1008 probes of Example 1. The liver, placenta and HeLa cDNA libraries were oligo dT primed, while the colon cDNA library was randomly primed. In each screening, $5x10^6$ phages were adsorbed to

Escherichia coli, strain Y1088, plated at a density of 40,000 p.f.u/15 cm petri dish and grown at 37°C for 18 hours. Nitrocellulose filters were overlaid in duplicates on the plates, then immersed in DNA-denaturing solution, transferred further to a neutralizing solution, and then dried in vacuum at 80°C and prehybridized to allow non-specific sites to be saturated with unlabelled DNA. The 1008 probes were ^{32}P-end-labelled, using the T4 polynucleotide kinase and applied to the filters in a solution containing 6 SSC (1 SSC corresponds to 0.15M NaCl and 0.015M sodium citrate), 10 "Denhardt's solution" (a mixture of Ficoll, polyvinylpyrrolidone and bovine serum albumin (Pentax Fraction V) in water, according to T. Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, N.Y., 1982 p. 448), 0.5% SDS (sodium dodecyl sulfate), and 100 µg/ml salmon sperm DNA. Hybridization was carried out for 18 hours at 50°C with the colon library and at 42°C with the other libraries. Unbound labelled probe was washed with a solution containing 3SSC at 25°C and then twice again either at 42°C or, for the colon library, at 50°C. Positive clones, identified by exposure of the filters to autoradiography, were picked up, purified and checked for hybridization to the 1009 and 1010 probes under those same conditions which were applied for the screening with the 1008 probes, except that the temperature of hybridization and washing was 30°C.

The results of the screening are summarized in Table I. Clones which hybridized with all three probes could be detected only in the placenta and colon libraries. In further analysis of the nucleotide sequence, only the clones picked up from the colon library (the only library which was randomly primed) were found indeed to code for TBP-I. These clones were designated C1, C2, C3, and C4.

Table I: Libraries screened for the TBP-I cDNA and the clones which were isolated

Vector	Library	Clone name	Temp. of hybridization 1008 C°	1009 C°	1010 C°
λgt11 cDNA oligo dT primed					
	Liver	-	-	-	-
	HeLa	-	-	-	-
	Placenta	17	50°	-	-
		19	50°	30°	30°
		131	60°	30°	30°
		133	50°	30°	30°
		152	50°	-	-
λgt11 cDNA randomly primed					
	Colon*	C1	60°	30°	30°
		C2	60°	30°	30°
		C3	60°	30°	30°
		C4	60°	30°	30°

*normal tissue around colon cancer

Example 3

Characterization of the isolated clones from the human colon cDNA library

The purified lambda gt11 DNA containing positive cDNA clones were digested with EcoR1 and size-fractionated on 1% agarose gel. Two of the clones had an insert size of about 1.0 Kb, the third was of 0.9 Kb and the fourth had two inserts of 0.9 and 0.8 Kb. Cross-hybridization among the four clones was tested by Southern blotting. The results are summarized in Table II. Clones C2 and C3 were found to contain the same 1.0 Kb insert. The restriction map of this insert is shown in Figure 1. Clone C5 contains two inserts, of 0.9 and 0.8 Kb: the 0.9 Kb insert constitutes part of the insert of C2, while the 0.8 Kb insert seems to be unrelated. Clone C1 also contains

a 0.9 Kb insert which constitutes part of the insert of C2.

Table II: Insert sizes and interrelationships in the various cDNA clones for TBP-I

cDNA clone	Insert size (Kb)	Cross hybridization: with the C1 insert	with the C2 insert	with the EcoRI-PstI 165 nucleotide fragment of C2
C1	0.9	++	+	++
C2	1.0	+	++	++
C3	1.0	+	++	++
C5	0.9, 0.8	+	++	++

The 1.0 Kb EcoRI insert of the C2 clone was subcloned in a Bluescript plasmid vector of Stratagene Cloning System (San Diego, Cal.) and E. coli TG1 competent bacteria were transformed therewith. The transformed bacteria were deposited with the Collection Nationale de Cultures de Microorganismes (C.N.C.M.), of the Institut Pasteur, Paris, France, on December 6, 1989 under the Budapest Treaty, and it was assigned the deposit number CNCM I-917.

Example 4

Screening of an oligo dT primed (human placenta) cDNA library with the DNA probes

To isolate sequences extending 3' to the insert of clone C2, $0.5x10^6$ recombinants from human placenta cDNA library in lambda gt11 were screened for hybridization with the aid of a probe prepared from the 125-nucleotide PstI-PstI fragment of clone C2 (see Fig. 1) which was labelled with the multiprime DNA labelling systems kit

(Amersham). The technique is based on the use of random sequence hexanucleotide to prime DNA synthesis on denatured template DNA at numerous sites along its length.

Phages were adsorbed to E.coli strain Y1088, plated at a density of 40,000 p.f.u/15 cm petri dish and grown at 37°C overnight. Two sets of nitrocellulose filters were overlaid and immersed in a tray containing DNA-denaturing solution. The filters were washed, fixed, neutralized, dried at 80°C under vacuum and prehybridized to allow non-specific sites to be saturated by unlabelled DNA. Then the filters were hybridized with the ^{32}P-labelled probe overnight at 65°C. Unbound label was washed first in a solution containing 1SSC and 0.1% SDS (twice at 25°C and then twice again at 65°C) and then at 65°C in a solution containing 0.1 SSC and 0.1% SDS. Filters were autoradiographed. Thirteen positive clones were obtained and picked up. After purification, these clones were tested for hybridization with a probe constructed from the C2 insert from which the above EcoRI-PstI insert was deleted. Four clones were found to hybridize. DNA from these positive clones was isolated after purifying the phages by centrifugation in a CsCl solution. Their inserts were excised by cutting with EcoRI and their sizes were estimated by electrophoresis on 1% agarose gel. The phage containing the largest fragment of about 2.0 Kb was subjected to further analysis. Its insert was subcloned in a Bluescript plasmid vector and E.coli TG 1 competent bacteria were transformed therewith.

Example 5

Determination of the nucleotide sequence in the cloned DNA

DNA of the 1.0 Kb EcoRI insert of the C2 clone propagated in the

Bluescript plasmid vector was subjected to partial degradation to various extents using the Erase-a-Base Progema kit. DNA of plasmids containing the insert at various degrees of degradation was denatured and subjected to sequencing as described by Hattoni and Sakaki (Anal. Biochem. 152, 232-238) with the aid of the Sequenase Kit (USB).

The insert was shown to have 965 nucleotides. Figure 2 shows a partial nucleotide sequence of the C2 insert and the deduced amino acid sequence which it codes for. Within this sequence the NH_2-terminal amino acid sequence of TBP-I, on the basis of which the cDNA had been cloned, can be detected (underlined). Figure 3 shows the 343-965 nucleotide sequence of the C2 insert and Figure 4 shows a possible total nucleotide sequence of the C2 insert.

Example 6

Detection and sizing of the mRNA for TBP-I by Northern blot analysis

Total RNA was extracted from cells of the U937 and HT29 lines by the "hot phenol" method according to T. Maniatis et al., op. cit., p. 194. Samples of 50 and 25μg RNA were analyzed by electrophoresis on 1.5% agarose gel in the presence of 2.2% formaldehyde followed by blotting to charged nylon filter. The EcoRI insert of the C2 clone was ^{32}P-labeled with the use of the multiprime DNA labelling systems kits and hybridized to the charged nylon blot (42°C in 50% formamide). As shown in Figure 5 in both cells the cDNA was found to hybridize to mRNA of just a single size, of about 19S-21S and having about 2500 nucleotides (right stronger dot - 50 μg RNA, left weaker dot - 25 μg RNA).

Claims

1. A DNA molecule comprising a recombinant DNA molecule or a cDNA molecule coding for a protein comprising the amino acid sequence of TBP-I or a protein substantially homologous therewith.

2. A cDNA molecule according to claim 1 comprising a 1.08 Kb C2 insert of about 0.965 Kb, said insert providing a partial restriction map substantially as shown in Figure 1 upon digestion with restriction enzymes.

3. A cDNA molecule according to claim 1 or 2 comprising the partial nucleotide sequence shown in Figure 2 or a nucleotide sequence substantially homologous therewith.

4. A cDNA molecule according to claim 1 or 2 comprising the partial nucleotide sequence shown in Figure 3 or a nucleotide sequence substantially homogolous therewith.

5. A cDNA molecule according to claim 1 or 2 comprising the partial nucleotide sequences shown in Figures 2 and 3 or nucleotide sequences substantially homogolous therewith.

6. A cDNA molecule according to any of the preceding claims comprising the nucleotide sequence shown in Figure 4 or a nucleotide sequence substantially homologous therewith.

7. A cDNA molecule according to any of the preceding claims

encoding a protein comprising the amino acid sequence of TBP-I or a protein substantially homologous therewith.

8 A replicable plasmid vector comprising a DNA molecule according to any of the preceding claims.

9. A replicable plasmid vector according to claim 8 comprising the C2 insert of any of claims 2 to 8.

10. A bacterium transformed with a replicable plasmid vector according to claim 8 or 9.

11. A bacterium according to claim 10 which is an E.coli strain.

12. E.coli TG1 C2 having the deposit number CNCM I-917.

13. Oligonucleotide probes useful for picking up genes from cDNA libraries which code for proteins comprising the amino acid sequence of TBP-I, said probes having the formula:

```
1008        GGI GTC CCI TTC ATA TAA GTA GGI GT
                  T       T   G   G   G
                                      T

1009        GGA GTC CCA TTC ATA TA
              C   T   C   T   G
              T       G
              G       T

1010                    TTC ATA TAA GTA GGA GT
                          T   G   G   G   C
                                  T       G
                                          T
```

14. DNA molecules hybridizable to all three oligonucleotide probes, according to claim 13 and which code for a protein comprising

the amino acid sequence of TBP-I or a protein substantially homologous therewith.

15. mRNA isolated from U937 and HT29 cells having a size of about 2.5 Kb and being hybridizable with the C2 insert of claims 2 to 7.

16. The mRNA of claim 15 coding for a protein comprising the amino acid sequence of TBP-I or a protein substantially homologous therewith.

For the Applicants:

Paulina Ben-Ami

Patent Attorney

FIGURE 1

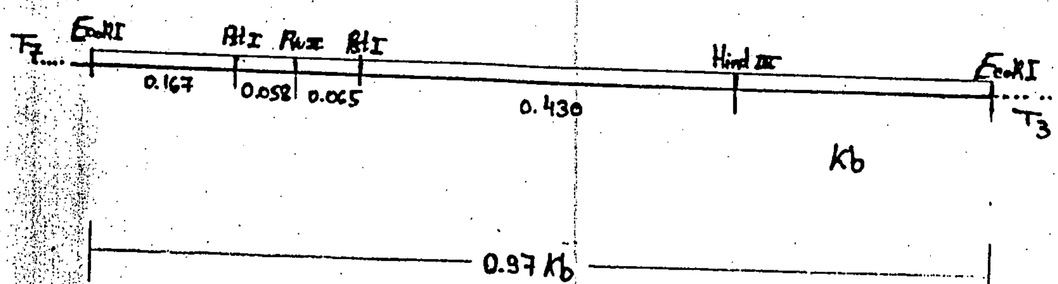

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Figure 2

```
    TATCGAATTCCGGTCCCTCACCTAGGGGACAGGGAGAAGAGAGATAGTGTCTGTCCCCAA
  1 ---------+---------+---------+---------+---------+---------+ 60
    ATAGCTTAAGGCCAGGGAGTGGATCCCCTGTCCCTCTTCTCTCTATCACAGACAGGGGTT
    TyrArgIleProValProHisLeuGlyAspArgGluLysArgAspSerValCysProGln

    GGAAAATATATCCACCCTCAAAATAATTCGATTTGCTGTACCAAGTGCCACAAAGGAACC
 61 ---------+---------+---------+---------+---------+---------+ 120
    CCTTTTATATAGGTGGGAGTTTTATTAAGCTAAACGACATGGTTCACGGTGTTTCCTTGG
    GlyLysTyrIleHisProGlnAsnAsnSerIleCysCysThrLysCysHisLysGlyThr

    TTCTTGTACAATGACTGTCCAGGCCCGGGGCAGGATACGGACTGCAGGGAG
121 ---------+---------+---------+---------+---------+-
    AAGAACATGTTACTGACAGGTCCGGGCCCCGTCCTATGCCTGACGTCCCTC
    PheLeuTyrAsnAspCysProGlyProGlyGlnAspThrAspCysArgGlu
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FIGURE 4

```
    TATCGAATTCCGGTCCCTCACCTAGGGGACAGGGAGAAGAGAGATAGTGTCTGTCCCCAA
  1 ---------+---------+---------+---------+---------+---------+
    ATAGCTTAAGGCCAGGGAGTGGATCCCCTGTCCCTCTTCTCTCTATCACACACAGGGCTT  60
    TyrArgIleProValProHisLeuGlyAspArgGluLysArgAspSerValCysProGln  -
    GGAAAATATATCCACCCTCAAAATAATTCGATTTGCTGTACCAAGTGCCACAAAGGAACC
 61 ---------+---------+---------+---------+---------+---------+
    CCTTTTATATAGGTGGGAGTTTTATTAAGCTAAACGACATGGTTCACGGTGTTTCCTTGG  120
    GlyLysTyrIleHisProGlnAsnAsnSerIleCysCysThrLysCysHisLysGlyThr  -
    TTCTTGTACAATGACTGTCCAGGCCCGGGGCAGGATACGGACTGCAGGGACTGTGAGAGC
121 ---------+---------+---------+---------+---------+---------+
    AAGAACATGTTACTGACAGGTCCGGGCCCCGTCCTATGCCTGACGTCCCTCACACTCTCG  180
    PheLeuTyrAsnAspCysProGlyProGlyGlnAspThrAspCysArgGluCysGluSer  -
    GGCTCCTTCACGCTTCAGAAACCACCTCAGACACTGCCTCAGCTGCTCCAGATGCCGAAA
181 ---------+---------+---------+---------+---------+---------+
    CCGAGGAAGTGCGAAGTCTTTGGTGGAGTCTGTGACGGAGTCGACGAGGTCTACGGCTTT  240
    GlySerPheThrLeuGlnLysProProGlnThrLeuProGlnLeuLeuGluMetProLys  -
    GGAATGGTCAGTGAGACTCTTCTTGCACAGTGGACCGGGACACCGTGTGTGGCTGCAGGA
241 ---------+---------+---------+---------+---------+---------+
    CCTTACCAGTCACTCTGAGAAGAACGTGTCACCTGGCCCTGTGGCACACACCGACGTCCT  300
    GlyMetValSerGluThrLeuLeuAlaGlnTrpThrGlyThrProCysValAlaAlaGly  -
    AGAACCAGTACCGGCATTATTGGAGTGAAAACCTTTTCCAGTGCTTCAATTGCAGCCTCT
301 ---------+---------+---------+---------+---------+---------+
    TCTTGGTCATGGCCGTAATAACCTCACTTTTGGAAAAGGTCACGAAGTTAACGTCGGAGA  360
    ArgThrSerThrGlyIleIleGlyValLysThrPheSerSerAlaSerIleAlaAlaSer  -
    GCCTCAATGGGACCGTGCACCTCTCCTGCCAGGAGAAACAGAACACCGTGTGCACCTGCC
361 ---------+---------+---------+---------+---------+---------+
    CGGAGTTACCCTGGCACGTGGAGAGGACGGTCCTCTTTGTCTTGTGGCACACGTGGACGG  420
    AlaSerMetGlyProCysThrSerProAlaArgArgAsnArgThrProCysAlaProAla  -
    ATGCGATTCTTTCTAAGAGAAAACGAGTGTGTCTCCTGTAGTAACTGTAAGAAAAGCCTG
421 ---------+---------+---------+---------+---------+---------+
    TACGCTAAGAAAGATTCTCTTTTGCTCACACAGAGGACATCATTGACATTCTTTTCGGAC  480
    MetArgPhePheLeuArgGluAsnGluCysValSerCysSerAsnCysLysLysSerLeu  -
    GAGTGCACGAAGTTGTGCCTACCCCAGATTGAGAATGTTAAGGGCACTGAGGACTCAGGC
481 ---------+---------+---------+---------+---------+---------+
    CTCACGTGCTTCAACACGGATGGGGTCTAACTCTTACAATTCCCGTGACTCCTGAGTCCG  540
```

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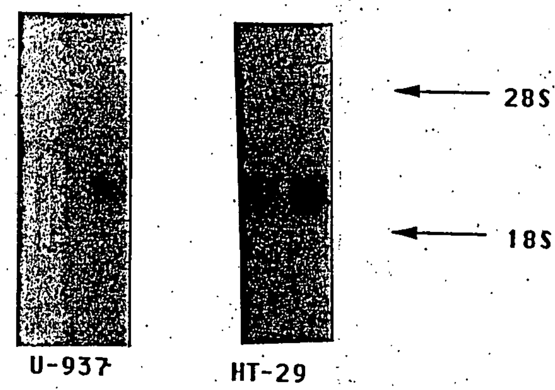
FIGURE 4 (contd.)

```
    GluCysThrLysLeuCysLeuProGlnIleGluAsnValLysGlyThrGluAspSerGly -
    ACCACAGTCGTGTTGAAAATGGTCATTTTCTTTGGTCTTTGCCTTTTATCCCTCCTCTTC
541 ---------+---------+---------+---------+---------+---------+ 600
    TGGTGTCAGCACAACTTTTACCAGTAAAAGAAACCAGAAACGGAAAATAGGGAGGAGAAG
    ThrThrValValLeuLysMetValIlePhePheGlyLeuCysLeuLeuSerLeuLeuPhe -
    ATTGGTTTAATGTATCGCTACCAACGGTGGAAGTCCAAGCTCTACTCCATTGTTTGTGGG
601 ---------+---------+---------+---------+---------+---------+ 660
    TAACCAAATTACATAGCGATGGTTGCCACCTTCAGGTTCGAGATGAGGTAACAAACACCC
    IleGlyLeuMetTyrArgTyrGlnArgTrpLysSerLysLeuTyrSerIleValCysGly -
    AAATCGACACTGAAAAGAGGGGAGCTTGAAGCAACTACTACTAAGCCCTGGCCCCAAACC
661 ---------+---------+---------+---------+---------+---------+ 720
    TTTAGCTGTGACTTTTCTCCCCTCGAACTTCGTTGATGATGATTCGGGACCGGGGTTTGG
    LysSerThrLeuLysArgGlyGluLeuGluGlyThrThrThrLysProTrpProGlnThr -
    CAAGCTTCAGTCCCACTCCAGGCTTCACCCCCACCCTGGGCTTCAGTCCCGTGCCCAGTT
721 ---------+---------+---------+---------+---------+---------+ 780
    GTTCGAAGTCAGGGTGAGGTCCGAAGTGGGGGTGGGACCCGAAGTCAGGGCACGGGTCAA
    GlnAlaSerValProLeuGlnAlaSerProProProTrpAlaSerValProCysProVal -
    CCACCTTCACCTCCAGCTCCACCTATACCCCCGGTGACTGTCCCAATTTCTGGCTCCCCG
781 ---------+---------+---------+---------+---------+---------+ 840
    GGTGGAAGTGGAGGTCGAGGTGGATATGGGGGCCACTGACAGGGTTAAAGACCGAGGGGC
    ProProSerProProAlaProProIleProProValThrValProIleSerGlySerPro -
    CAGAGAGGTGGCACCACCTATCAGGGGGCTGACCCCATCCTTGCGACAGCCCTCGCCTCC
841 ---------+---------+---------+---------+---------+---------+ 900
    GTCTCTCCACCGTGGTGCATAGTCCCCCGACTGCGGTAGGAACGCTGTCGGGAGCGGAGG
    GlnArgGlyGlyThrThrTyrGlnGlyAlaAspProIleLeuAlaThrAlaLeuAlaSer -
    GACCCCATCCCCAACCCCCTTCAGAAGTGGGAGGACAGCGCCACAAGCCACAGAGCCCGG
901 ---------+---------+---------+---------+---------+---------+ 960
    CTGGGGTAGGGGTTGGGGGAAGTCTTCACCCTCCTGTCGCGGTGTTCGGTGTCTCGGGCC
    AspProIleProAsnProLeuGlnLysTrpGluAspSerAlaThrSerHisArgAlaArg -
    AATTC
961 ----- 965
    TTAAG
    Asn??? -
```

FIGURE 5

Detection of TBPI mRNA by northern blot hybridization



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